

Safety and Immunogenicity of Seven Dosing Regimens of the Candidate RTS,S/AS01_E Malaria Vaccine Integrated Within an Expanded Program on Immunization Regimen

A Phase II, Single-Center, Open, Controlled Trial in Infants in Malawi

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Background: In a phase III trial, the RTS,S/AS01 malaria vaccine produced lower anti-circumsporozoite (CS) antibody titers when co-administered with Expanded Programme on Immunization vaccines (0-, 1- and 2-month schedule) at 6 to 12 weeks compared with 5 to 17 months at first vaccination. Alternative infant immunization schedules within the Expanded Programme on Immunization were investigated.

Methods: This phase II, open, single-site (Blantyre, Malawi) trial was conducted in infants 1 to 7 days of age. Subjects were equally randomized across 7 groups to receive 3 doses of RTS,S/AS01_E at time points that included ≤7 days, 6, 10, 14 and 26 weeks, and 9 months. All RTS,S/AS01_E groups plus a control group (without RTS,S/AS01_E) received Bacillus Calmette-Guérin + oral poliovirus vaccine at ≤7 days, diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae type b* vaccine + oral poliovirus vaccine at 6, 10, and 14 weeks and measles vaccine at 9 months; one RTS,S/AS01_E group and the control additionally received hepatitis B vaccination at ≤7 days. Serum anti-CS antibody geometric mean concentration (GMC; enzyme-linked immunosorbent assay) and safety were assessed up to age 18 months.

Results: Of the 480 infants enrolled, 391 completed the study. No causally related serious adverse event was reported. A higher frequency of fever within 7 days of RTS,S/AS01_E vaccination compared with control

was observed. Compared with the standard 6-, 10-, 14-week schedule, anti-CS antibody GMC ratios post-dose 3 were significantly higher in the 10-, 14- and 26-week group only (ratio 1.80; 95% confidence interval, 1.24–2.60); RTS,S/AS01_E vaccination at ≤7 days and 10 and 14 weeks produced significantly lower anti-CS GMCs (ratio 0.59; 95% confidence interval, 0.38–0.92).

Conclusions: Initiation of RTS,S/AS01_E vaccination above 6 weeks of age tended to improve anti-CS antibody responses. Neonatal vaccination was well tolerated but produced a comparatively lower immune response.

Key Words: RTS,S/AS01; *falciparum*; malaria; immunogenicity; neonates

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FOCUS ON STUDY PARTICIPANT SECTION

The RTS,S/AS01_E malaria vaccine has consistently shown protection against clinical malaria episodes in infants and children when administered with or without concurrent Expanded Programme on Immunization (EPI) vaccines. The results of the present study indicate that administration of the first dose of RTS,S/AS01_E as a 3-dose schedule in co-administration with EPI vaccines at a slightly older age—10 weeks instead of 6 weeks—in the infant population is well tolerated and improves immunogenicity. This study shows the potential to optimize the immune response induced by RTS,S/AS01_E by using different immunization schedules within the currently existing immunization visits. Further study is required on the feasibility of co-administration, and potential benefit in terms of efficacy, of RTS,S/AS01_E vaccination at a later age within the EPI schedule. Neonatal vaccination with RTS,S/AS01_E was comparatively less immunogenic.

Malaria causes devastating morbidity and mortality, with an estimated 214 million malaria cases and 438,000 malaria deaths occurring worldwide in 2015.¹ Malaria mortality occurs predominantly in children, with an estimated 306,000 deaths in children younger than 5 years of age each year, of which 95% occur in Africa.

The development of efficacious malaria vaccines has been identified as a key component of a sustainable malaria control program, with consequent benefits for health and the economy.² GSK Vaccines' pre-erythrocytic *Plasmodium falciparum* malaria vaccine, RTS,S/AS01_E, consists of sequences of the circumsporozoite (CS) protein fused to hepatitis B surface antigen (HBsAg) adjuvanted with AS01 (liposome formulation with MPL (3-*O*-desacyl-4'-monophosphoryl lipid A; produced by GSK) and QS-21 (*Quillaja saponaria* Molina, fraction 21; Licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation) immunostimulants).³

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The Expanded Programme on Immunization (EPI) is an established program that uses immunization regimens aimed at maximizing immunization coverage to address disease burden early in life.

During phase II development, lower anti-CS antibody titers were produced when an earlier formulation of the vaccine (RTS,S/AS02) and the RTS,S/AS01_E formulation were co-administered with EPI vaccines on a 0-, 1- and 2-month schedule to infants 6 to 12 weeks of age at first vaccination, compared with children 1 to 4 years of age⁴⁻⁸; this difference in immunogenicity did not appear to result in a difference in protective efficacy. Although an immature immune system or an inhibitory effect of maternally derived anti-CS antibodies may have played a role, interference of co-administered routine EPI vaccines may also have contributed to the lower immunogenicity observed in young infants.⁹

The current study was designed to investigate alternative vaccination schedules to improve anti-CS immune responses of RTS,S/AS01_E in infants when integrated within an EPI regimen in Blantyre, Malawi. Although no correlate of protection has been established, a link between higher anti-CS titers and decreased risk of infection has been shown in some trials.^{4,6} The exploratory schedules comprised administration of 3 doses of RTS,S/AS01_E between birth (neonatal dose) and 9 months of age, using different existing contacts with the health service. In previous trials with GSK Vaccines' hepatitis B vaccine, it was observed that increasing the interval between the second and third dose enhances humoral response.^{10,11} In this study, administration of the third dose of RTS,S/AS01_E at 26 weeks of age was investigated to assess if increased spacing of RTS,S/AS01_E doses improves anti-CS antibody responses.

Starting hepatitis B immunization at birth or in the neonatal period, as recommended by World Health Organization,¹² is desirable to control mother to infant transmission of hepatitis B infection and the subsequent development of chronic carriage. Furthermore, previous phase II trials indicated that prior hepatitis B vaccination may "prime" for anti-CS antibody response.^{13,14} Thus, a schedule including a neonatal dose of a hepatitis B vaccine (*Engerix-B**) was investigated.

Potential inhibition of RTS,S/AS01_E-specific immune responses after co-administration with diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae type b* vaccine (DTPw-HepB/Hib) was evaluated by the inclusion of schedules in which doses of RTS,S/AS01_E were not co-administered with DTPw-HepB/Hib (*Tritanrix HepB/Hib**). These schedules also investigated any effect on anti-CS antibody response of age at first dose of RTS,S/AS01_E.

Although there is currently no scheduled visit at 6 months of age in the EPI, at this time point, parents of children in Africa are requested to bring their child to their local health facility for administration of Vitamin A. An exploratory vaccination schedule within existing EPI visits, but outside the EPI DTPw-HepB/Hib vaccination schedule, was also investigated, given the substantial number of antigens currently administered at these time points and possible future increase in co-administered antigens. In this schedule, the first dose of RTS,S/AS01_E was co-administered with the third dose of DTPw-HepB/Hib, the second dose without co-administration but coinciding with a Vitamin A EPI visit and the third dose in co-administration with the measles vaccine (*Rouvax**).

The primary aims of the study were to compare CS antigen immunogenicity of RTS,S/AS01_E between exploratory schedules and the reference schedule at 6, 10, 14 weeks of age and to evaluate safety over an 18-month period.

**Engerix-B*, *Tritanrix*, and *Hiberix* are trademarks of the GSK group of companies. *Rouvax* is a trademark of Sanofi Pasteur.

MATERIALS AND METHODS

A summary of the protocol is available at www.gsk-clinical-studyregister.com (GSK study 111315).

Ethics

The study was registered with the Pharmacy, Medicines and Poisons Board, Malawi, and at ClinicalTrials.gov (ClinicalTrials.gov number: NCT01231503). Approval was obtained from the National Health Sciences Research Committee, Malawi, the College of Medicine Research Ethics Committee, University of Malawi, and the Research Ethics Sub-committee for Physical Interventions, University of Liverpool, United Kingdom. The trial was conducted in accordance with the provisions of the International Conference on Harmonization and Good Clinical Practice guidelines.

Recruitment of Study Subjects

Before study start, activities were carried out to raise awareness among health professionals in Blantyre district, as well as at different levels of the community.

Pregnant women who were interested in participating in the study were identified at the antenatal clinic at the Bangwe Health Centre, Blantyre, Malawi. Potential recruits were required to be resident in the Bangwe study clinic catchment area for the foreseeable future, a minimum age of 18 years at the time of delivery, in the third trimester of pregnancy (27 to 42 weeks) and to provide informed consent for antenatal screening for hepatitis B and human immunodeficiency virus infection. Written informed consent for subject participation was requested from mothers antenatally, as ideally cord blood was taken for screening purposes. If consent was given >7 days before procedures being carried out on the child, a brief re-consenting process was performed.

Only infants whose mothers were negative for carriage of the hepatitis B virus and human immunodeficiency virus infection were eligible to be screened for the trial. Eligibility criteria included male or female infants between 1 and 7 days of age (inclusive), born at full term (gestation period between 37 and 42 weeks) confirmed by Dubowitz score¹⁵ and with a minimum weight of 2.5 kg (see Appendix, Section 1, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). Subjects had to be healthy as determined by physical examination, medical history records and laboratory screening tests of hematology and renal and hepatic function. For participation in the study, no previous vaccinations with diphtheria, tetanus, pertussis (whole-cell or acellular), *Haemophilus influenzae type b*, hepatitis B, Bacillus Calmette-Guérin (BCG) tuberculosis, measles or oral polio vaccines were allowed.

Study Design

The study was a phase II, open, randomized, controlled trial with 8 groups. All groups received standard EPI vaccines. Seven groups additionally received RTS,S/AS01_E as a 3-dose schedule with or without neonatal hepatitis B vaccine. The control group additionally received a neonatal dose of hepatitis B vaccine (Fig. 1). The study duration was approximately 18 months per child.

Study Vaccines, Vaccination, Randomization

RTS,S/AS01 is manufactured by GSK Vaccines (Rixensart, Belgium). Each 0.5 mL RTS,S/AS01 dose as tested in this study contained RTS,S (25 µg) and AS01_E, an Adjuvant System containing 25 µg MPL (3-*O*-desacyl-4'-monophosphoryl lipid A) and 25 µg QS-21 (*Quillaja saponaria* Molina, fraction 21)³ RTS,S/AS01 was administered intramuscularly in the left antero-lateral thigh.

EPI vaccines comprised a diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae type b* pentavalent vaccine (*Tritanrix HepB/Hib*; GSK Vaccines), an oral polio-virus vaccine containing serotypes 1, 2 and 3 (*Polio Sabin**; GSK

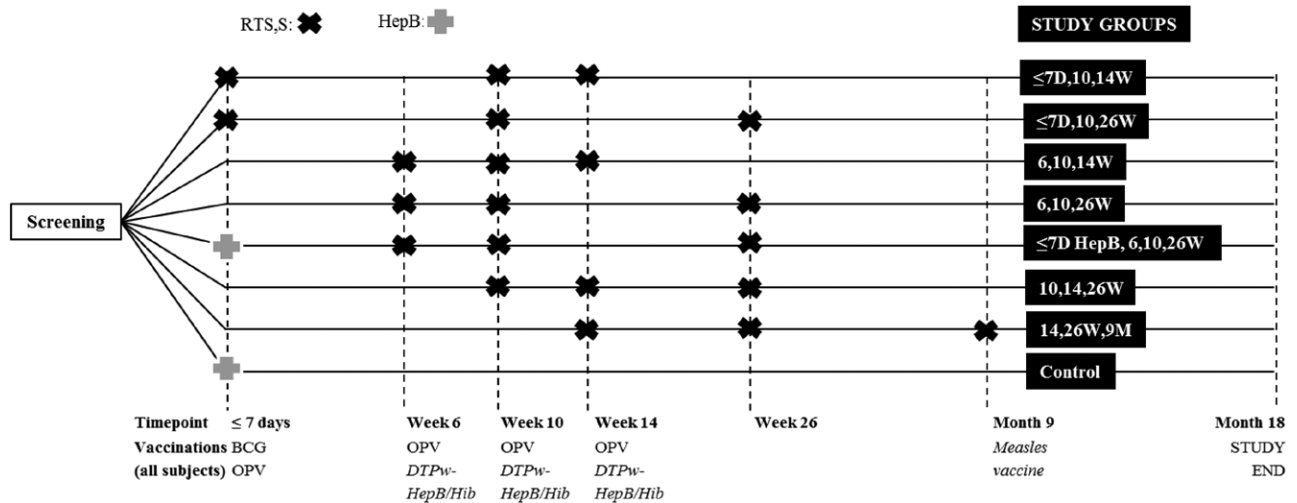


FIGURE 1. Study design overview: study groups, vaccinations. ≤7D, 10, 14W group indicates RTS,S/AS01_E at ≤7 days, 10 and 14 weeks; ≤7D, 10, 26W group, RTS,S/AS01_E at ≤7 days, 10 and 26 weeks; 6, 10, 14W group, RTS,S/AS01_E at 6, 10 and 14 weeks; 6, 10, 26W group, RTS,S/AS01_E at 6, 10 and 26 weeks; ≤7D HepB, 6, 10, 26W, HepB vaccine at ≤7 days and RTS,S/AS01_E at 6, 10 and 26 weeks; 10, 14, 26W group, RTS,S/AS01_E at 10, 14 and 26 weeks; 14, 26W, 9M, RTS,S/AS01_E at 14, 26 weeks and 9 months; BCG, *Bacillus Calmette-Guérin* (BCG) (tuberculosis) vaccine (Statens Serum Institute and Biovac Institute Commercial BCG); Control, HepB vaccine at ≤7 days; DTPwHepB/Hib, diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae type b* vaccine (Tritanrix HepB/Hib, GSK Vaccines); HepB, Hepatitis B vaccine (Engerix-B); Measles vaccine, attenuated measles vaccine (Rouvax, Sanofi); OPV, oral poliovirus vaccine (Polio Sabin, GSK Vaccines); RTS,S, RTS,S/AS01_E.

Vaccines), a hepatitis B vaccine (HepB; *Engerix-B*; GSK Vaccines), a BCG vaccine (Statens Serum Institute) and an attenuated measles vaccine (*Rouvax*, Sanofi Pasteur). Details for the EPI vaccines and administration are provided in Appendix, Section 2, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>.

Treatment allocation was performed at the investigator site using a standard Statistical Analysis System (SAS) programmed randomization list generated at GSK Vaccines, Belgium. Randomization was not stratified, but ensured an equal number distribution of subjects across treatment groups. The sample size of this descriptive phase II trial was powered to detect at least a 2-fold difference in geometric mean titer (GMT) between any 2 groups.

**Polio Sabin* is a trademark of the GSK group of companies.

Immunogenicity Assessments

Immunology assessments are described in detail in Appendix Section 3, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>. Briefly, anti-CS repeat region immunoglobulin G antibodies were measured by standard enzyme-linked immunosorbent assay methodology using plate-adsorbed R32LR antigen [NVD(P(NANP)₁₅]₂LR, as previously described.¹⁶ Antibodies against hepatitis B surface antigen (anti-HBs) were measured by chemiluminometric immunoassay (as measured by the Siemens Centaur XP CLIA).

Safety Assessments

As this was the first trial of the RTS,S/AS01_E candidate malaria vaccine in neonates, a rigorous safety monitoring plan was put in place (see Appendix, Section 4, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). The study was overseen by an Independent Data Monitoring Committee operating under a charter and assisted by 2 Local Safety Monitors.

The local injection site and general solicited adverse events (AEs) were monitored over 7 days after each vaccination (Days 0 to 6) and were graded as mild, moderate or severe (grades 1, 2 and 3, respectively). All other AEs (unsolicited) were recorded

over a 30-day (Days 0 to 29) period after each vaccination. Serious adverse events (SAEs) were captured throughout the study. All injection site AEs were considered causally related to vaccination; the causality of all other AEs was assessed by the investigator. Hematologic and biochemical tests for safety assessment were conducted at various time points during the study.

At the time of protocol development, 2 SAEs of febrile seizure within 7 days of vaccination in 2 previous phase II trials were considered to be causally related to vaccination with RTS,S/AS01_E.^{8,14} In the large phase III trial,^{9,17,18} an imbalance of meningitis cases of any etiology (ie, including cases with and without confirmed etiology) was observed in children 5 to 17 months of age at first dose. Consequently, any febrile seizure occurring within 30 days of vaccination and any case of meningitis occurring during the study were to be recorded as SAEs. See Appendix, Sections 4 and 5.2, Supplemental Digital Content 1, <http://links.lww.com/INF/C993> for additional details on safety assessments, including assessment of AEs of specific interest.

Statistical Analysis

All analyses were conducted according to a predefined analysis plan. The co-primary endpoints of the study were anti-CS antibody concentrations at 1 month post-dose 3 of RTS,S/AS01_E and occurrence of SAEs from study start until month 10. A second analysis included occurrence of SAEs from study start until study end (month 18; tertiary objective).

The Total Vaccinated Cohort (TVC) included all subjects who were randomized and received a dose of BCG vaccine. All safety analyses were performed on the TVC population. The According To Protocol cohort for immunogenicity included all subjects in the TVC who received all vaccinations according to protocol procedures within specified intervals and did not take any immune-modifying medication or have blood transfusions.

Seropositivity rates for CS (≥0.5 EU/mL), pertussis (≥15 ELU/mL) and measles (≥150 mIU/mL) and seroprotective rates

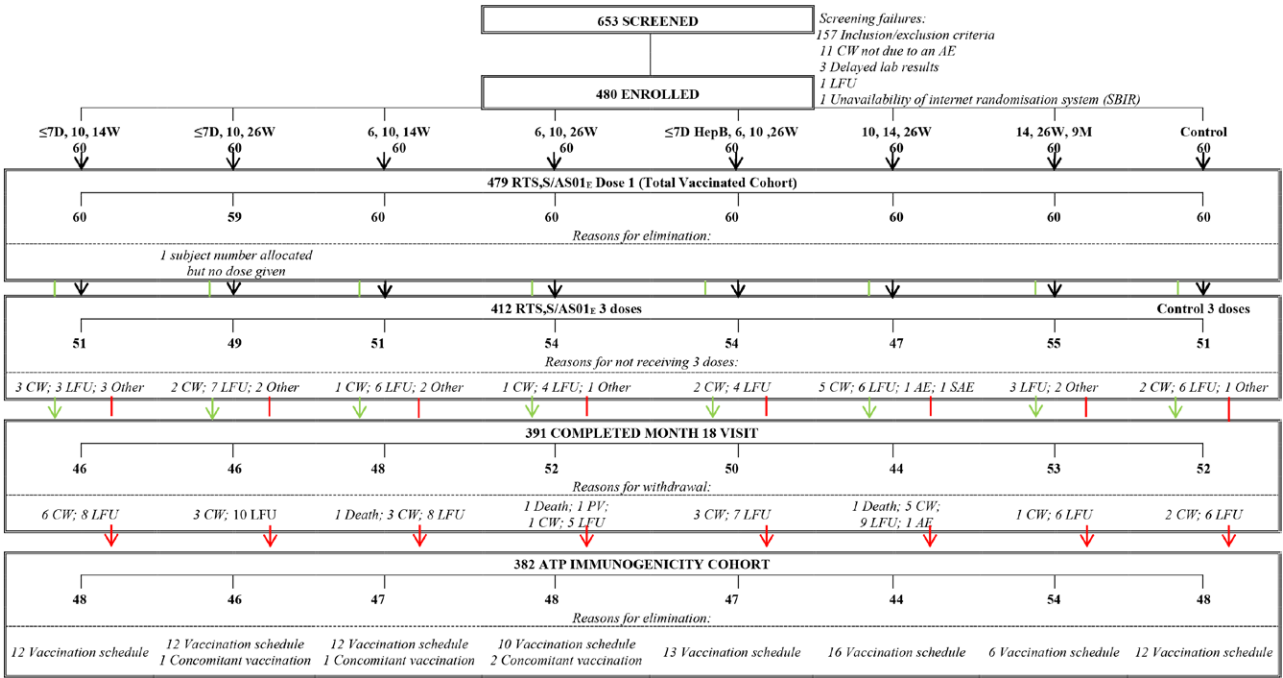


FIGURE 2. CONSORT diagram for study participants. ≤7D, 10, 14W indicates RTS,S/AS01_E at ≤7 days, 10 and 14 weeks; ≤7D, 10, 26W, RTS,S/AS01_E at ≤7 days, 10 and 26 weeks; 6, 10, 14W, RTS,S/AS01_E at 6, 10 and 14 weeks; 6, 10, 26W, RTS,S/AS01_E at 6, 10 and 26 weeks; ≤7D HepB, 6, 10, 26W, HepB vaccine at ≤7 days, and RTS,S/AS01_E at 6, 10 and 26 weeks; 10, 14, 26W, RTS,S/AS01_E at 10, 14 and 26 weeks; 14, 26W, 9M, RTS,S/AS01_E at 14, 26 weeks and 9 months; AE, adverse event; Completed, number of subjects who completed last study visit; Control, HepB vaccine at ≤7 days; CW, consent withdrawal (not due to an AE) or parent withdrew child; Enrolled, number of subjects who were enrolled in the study; Withdrawn, number of subjects who did not come back for the last visit; LFU, lost to follow up (subjects with complete/incomplete vaccination course) or migrated; PV, protocol violation; SAE, serious adverse event. [full color online](#)

for hepatitis Bs (≥10 mIU/mL), diphtheria (≥0.1 IU/mL), polyribosylribitol phosphate (purified capsular polyside of *Haemophilus influenzae* type b; ≥0.15 µg/mL), polio (≥8 ED₅₀ [endpoint dilution 50%]) and tetanus (≥0.1 IU/mL) together with geometric mean concentrations (GMCs)/GMTs plus exact 95% confidence intervals (CI) were evaluated. GMC ratios relative to the 6-, 10- and 14-week regimen were assessed. All safety analyses were performed by group and pooled neonatal groups in which safety was evaluated after neonatal dose 1 (pooled RTS,S: ≤7-day, 10-week and 14-weeks and ≤7-day, 10-week and 26-week groups; pooled HepB: ≤7-day HepB and 6-, 10-, 26- weeks and control groups). Analyses of safety after 3 doses of RTS,S/AS01_E or DTPw-HepB/Hib alone enabled comparison of equal follow up and number of vaccination visits; age at vaccination and number of doses of RTS,S/AS01_E co-administered with DTPw-HepB/Hib differed. Statistical analyses were conducted using SAS version 8 (SAS, Cary, NC), and further details are available in Appendix, Section 5, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>.

RESULTS

Study Population

Of the 480 subjects enrolled at birth, 479 were vaccinated, of whom 391 completed the study. The main reasons for withdrawal of the 88 subjects were consent withdrawal and migration/lost to follow up (Fig. 2).

All infants were less than 7 days old at the time of neonatal vaccination with BCG (Screening visit). An equal proportion of

males and females were enrolled (Table 1, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>).

Immunogenicity Outcomes

Post-dose 3 anti-CS, GMCs ranged from 128.2 to 392.6 EU/mL across all RTS,S/AS01_E co-administration schedules (Fig. 3). Anti-CS antibody responses persisted to month 18 in all 7 RTS,S/AS01_E schedules.

In subjects vaccinated at 6, 10 and 14 weeks of age (ie, the reference group for evaluation of anti-CS antibody response), the anti-CS antibody GMC post-dose 3 was 218.3 EU/mL (95% CI, 160–298). A higher anti-CS antibody GMC ratio was observed post-dose 3 only in the 2 groups where vaccination with RTS,S/AS01_E started after 6 weeks of age, that is, in the 10-, 14- and 26-week and 14-week, 26-week, 9-month groups, with a statistically significant increase in the 10-, 14- and 26-week schedule only (GMC ratio 1.80; 95% CI, 1.24–2.60) as compared with the reference schedule (6, 10 and 14 weeks in co-administration with DTPw-HepB/Hib + oral poliovirus vaccine [OPV]; Table 1).

Relatively lower anti-CS responses post-dose 3 were observed in groups receiving the first dose of RTS,S/AS01_E within 7 days of birth (≤7-day, 10-week and 14-week group: 128 EU/mL [95% CI, 92–178]; ≤7-day, 10-week and 26-week group: 137 EU/mL [95% CI, 93–201]), which was statistically significant compared with vaccination at 6, 10 and 14 weeks of age in the ≤7-day, 10-week and 14-week group (GMC ratio 0.59; 95% CI, 0.38–0.92).

Increasing the interval from 1 month to 3 months between doses 2 and 3 of RTS,S/AS01_E did not increase anti-CS response (post-dose 3 anti-CS antibody GMC ratios: ≤7-day, 10-week and

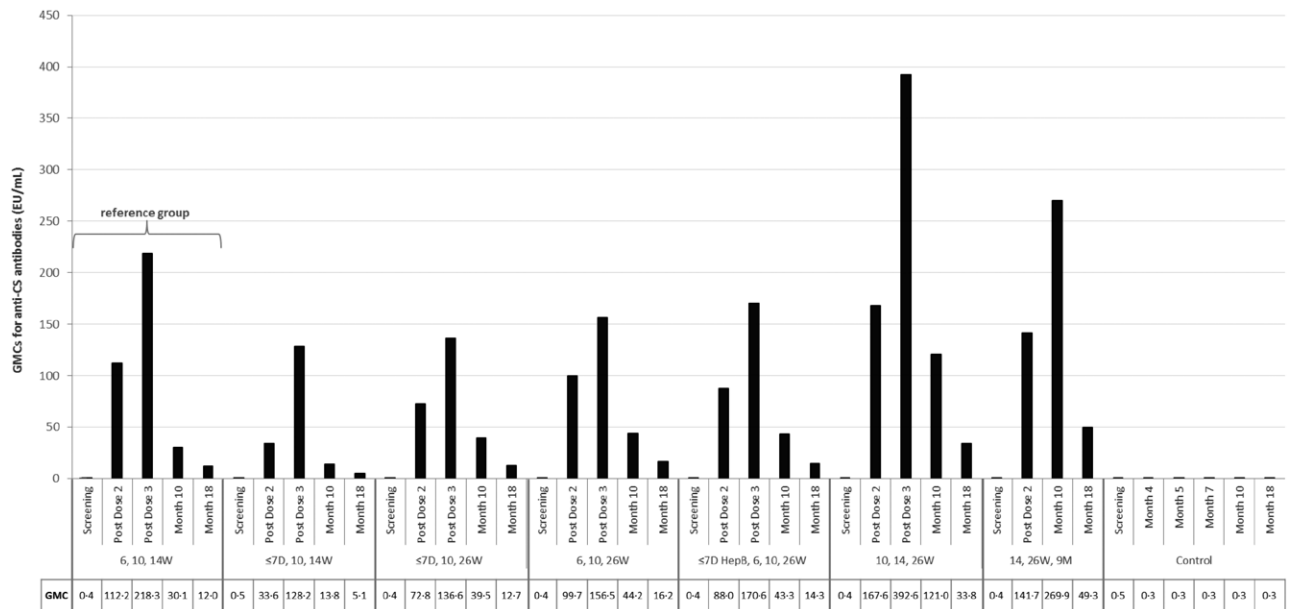


FIGURE 3. GMCs for anti-CS antibody concentration at each time point by group (ATP cohort for immunogenicity). 6, 10, 14W indicates RTS,S/AS01_E at 6, 10 and 14 weeks; ≤7D, 10, 14W, RTS,S/AS01_E at ≤7 days, 10 and 14 weeks; ≤7D, 10, 26W, RTS,S/AS01_E at ≤7 days, 10 and 26 weeks; 6, 10, 26W, RTS,S/AS01_E at 6, 10 and 26 weeks; ≤7D HepB, 6, 10, 26W, HepB vaccine at ≤7 days, and RTS,S/AS01_E at 6, 10 and 26 weeks; 10, 14, 26W, RTS,S/AS01_E at 10, 14 and 26 weeks; 14, 26W, 9M, RTS,S/AS01_E at 14, 26 weeks and 9 months; Control, HepB vaccine at ≤7 days; GMC, geometric mean concentration.

14-week group versus ≤7-day, 10-week and 26-week group, 0.94 [95% CI, 0.57–1.54]; 6-, 10- and 14-week group versus 6-, 10- and 26-week group, 1.39 [95% CI, 0.82–2.38]).

A neonatal dose of a hepatitis B vaccine did not increase the anti-CS response (post-dose 3 anti-CS antibody GMT ratio: 6-, 10-, and 26-week group versus HepB ≤7-day, 6-, 10-, 26-week group, 0.92 [95% CI, 0.51–1.66]).

One month after a single dose of measles vaccine at month 9, there was a trend for lower anti-measles antibody GMCs with RTS,S/AS01_E co-administration (14- and 26-week and 9-month group), though similar seropositivity rates were observed with or without (control group) co-administration of RTS,S/AS01_E (14- and 26-week and 9-month group: 92.3% [95% CI, 81.5–97.9] seropositive, GMC 1018 mIU/mL [95% CI, 752–1377]; control group: 89.1% [95% CI, 76.4–96.4] seropositive, GMC 1431 mIU/mL [95% CI, 997–2053]). Antibody responses to pertussis, diphtheria, polyribosylribitol phosphate, tetanus, OPV and measles antigens

are summarized in Table 10, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>.

All RTS,S/AS01_E regimens induced high anti-HBs (hepatitis B surface antigen) antibody responses (Fig. 1, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>); 100% of subjects were seroprotected for anti-HBs antibodies at all time points assessed up to month 18. In the control group receiving a hepatitis B vaccine as a neonatal dose followed by 3 doses of DTPw-HepB/Hib at 6, 10 and 14 weeks of age (current standard of care), 100%, 97.1% and 88.1% of subjects were seroprotected for anti-HBs antibodies at months 5, 10 and 18, respectively.

Safety Outcomes

Serious AEs

The proportion of subjects who experienced at least 1 SAE from study start until month 10 (primary safety endpoint) ranged across study groups from 5.0% to 16.7% (Table 2; Table 3,

TABLE 1. Anti-CS Antibody GMC Ratio Post-Dose 3 in Each Study Group Compared with 6-, 10-, 14-Week Group (ATP Cohort for Immunogenicity)

RTS,S/AS01 Group*	N	Anti-CS Antibody GMC			Anti-CS Antibody GMC Ratio vs 6, 10, 14W		
		GMC	95% CI		GMC Ratio	95% CI†	
6, 10, 14W	45	218.3	160.1	297.6	—		
6, 10, 26W	46	156.5	100.4	244.0	0.72	0.42	1.22
≤7 days, 10, 14W	47	128.2	92.2	178.2	0.59	0.38	0.92
≤7 days, 10, 26W	43	136.6	93.0	200.7	0.63	0.39	1.02
≤7 days HepB, 6, 10, 26W	43	170.6	114.6	254.1	0.78	0.48	1.28
10, 14, 26W	41	392.6	323.3	476.7	1.80	1.24	2.60
14, 26W, 9M	47	269.9	183.3	397.5	1.24	0.76	2.02

*See Figure 1 for study groups and vaccinations.

†95% CI for the GMC ratio (analysis of variance model – pooled variance).

GMC indicates geometric mean antibody concentration; N, number of subjects with postvaccination results available post-dose 3; numbers will differ to ATP cohort for immunogenicity.

TABLE 2. Safety Outcomes After 18 Months' Surveillance

Study groups*		Number (%) Infants Reporting Symptoms								Pooled neonatal	
		≤7D, 10, 14W	≤7D, 10, 26W	6, 10, 14W†	6, 10, 26W	≤7D HepB, 6, 10, 26W	10, 14, 26W	14, 26W, 9M	Control	RTS,S	HepB
		N ¹ = 60	N ¹ = 59	N ¹ = 54	N ¹ = 57	N ¹ = 57	N ¹ = 52	N ¹ = 57	N ¹ = 52	N ² = 119	N ² = 120
Solicited injection site symptoms during 7-day postvaccination period‡											
Pain	RTS,S/AS01 _E	All	2 (1.2)	4 (2.5)	8 (5.1)	4 (2.4)	2 (1.2)	1 (0.7)	0	—	5 (4.2)
		Grade 3§	0	0	0	0	0	0	—	0	—
	Control	All	7 (6.8)	2 (3.7)	7 (4.5)	11 (9.9)	3 (2.7)	4 (3.9)	1 (1.8)	12 (7.8)	3 (2.5)
Redness	RTS,S/AS01 _E	All	2 (1.2)	4 (2.5)	7 (4.5)	5 (3.0)	2 (1.2)	1 (0.7)	0	—	5 (4.2)
		Grade 3§	0	0	0	0	0	0	—	0	—
	Control	All	6 (5.8)	1 (1.9)	6 (3.8)	5 (4.5)	5 (4.4)	4 (3.9)	2 (3.5)	10 (6.5)	7 (5.9)
Swelling	RTS,S/AS01 _E	All	5 (3.1)	4 (2.5)	8 (5.1)	5 (3.0)	3 (1.8)	1 (0.7)	0	—	5 (4.2)
		Grade 3§	0	0	0	0	0	0	—	0	—
	Control	All	10 (9.7)	1 (1.9)	9 (5.8)	12 (10.8)	8 (7.1)	6 (5.9)	2 (3.5)	12 (7.8)	6 (5.0)
Solicited general symptoms during 7-day postvaccination period											
Drowsiness	All	0	0	0	0	0	0	1 (0.6)	0	0	1 (0.8)
	Grade 3§	0	0	0	0	0	0	0	0	0	0
Irritability	All	7 (4.3)	3 (1.9)	10 (6.4)	2 (1.2)	3 (1.8)	5 (3.4)	5 (3.0)	5 (3.2)	1 (0.8)	0
	Grade 3§	0	0	1 (0.6)	0	0	0	0	1 (0.6)	0	0
Loss of appetite	All	0	0	1 (0.6)	0	1 (0.6)	0	2 (1.2)	1 (0.6)	0	0
	Grade 3§	0	0	0	0	0	0	0	0	0	0
Fever	≥38.0°C	28 (17.2)	24 (14.8)	26 (16.7)	17 (10.3)	16 (9.6)	24 (16.3)	23 (13.6)	15 (9.7)	17 (14.3)	8 (6.7)
	>39.0°C§	0	1 (0.6)	1 (0.6)	0	0	1 (0.7)	2 (1.2)	0	0	0
Unsolicited (spontaneously reported) symptoms during 30-day postvaccination period											
All	All	36 (60.0)	35 (59.3)	28 (51.9)	29 (50.9)	35 (61.4)	36 (69.2)	47 (82.5)	31 (59.6)	26 (21.8)	33 (27.5)
	Grade 3§	1 (1.7)	0	1 (1.9)	1 (1.8)	2 (3.5)	2 (3.8)	1 (1.8)	0	0	0
	Related	0	0	0	1 (1.8)	0	0	0	0	0	0
SAEs (months 0–18)		Number (%) infants reporting serious adverse events after all doses of study vaccine									
		≤7D, 10, 14W	≤7D, 10, 26W	6, 10, 14W	6, 10, 26W	≤7D HepB, 6, 10, 26W	10, 14, 26W	14, 26W, 9M	Control	≤7D RTS	≤7D HepB
		N ² = 60	N ² = 59	N ² = 60	N ² = 60	N ² = 60	N ² = 60	N ² = 60	N ² = 60	N ² = 119	N ² = 120
All		7 (11.7)	5 (8.5)	6 (10.0)	9 (15.0)	8 (13.3)	12 (20.0)	5 (8.3)	4 (6.7)	2 (1.7)	3 (2.5)

Pooled neonatal RTS,S groups (≤7-days, 10-, 14-week and ≤7-days, 10-, 26-week groups). Pooled neonatal HepB vaccine groups (HepB vaccine at ≤7 days, RTS,S/AS01_E at 6, 10 and 26 weeks and Control [HepB vaccine at ≤7 days] groups).

N¹ indicates Number of subjects receiving 3 doses of RTS,S/AS01_E or DTPw-HepB/Hib alone for control; analyses of safety after 3 doses of RTS,S/AS01_E or DTPw-HepB/Hib alone enabled comparison of equal follow up and number of vaccination visits (age at vaccination and number of doses of RTS,S/AS01_E co-administered with DTPw-HepB/Hib differed); and N², Total Vaccinated Cohort.

*See Figure 1 for study groups and vaccinations.

†Standard 6-, 10- and 14-week reference schedule.

‡All solicited injection site symptoms were considered related to vaccination.

§Grade 3 AEs were defined as follows: pain, cries when limb is moved/spontaneously painful; redness/swelling, diameter >20 mm; drowsiness/irritability/unsolicited AEs, events preventing normal activity; loss of appetite, not eating at all; fever, temperature >39°C.

Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). Two subjects (1.7%) reported SAEs after a neonatal dose of RTS,S/AS01_E and 3 subjects (2.5%) after a neonatal dose of a hepatitis B vaccine. Over 18 months' surveillance, no safety concern was apparent across the different groups (Table 4, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). Three fatal SAEs were reported: respiratory distress (6-, 10- and 14-week group), acute respiratory distress syndrome (10-, 14- and 26-week group) and drowning (6-, 10- and 26-week group). No SAE was considered by the investigator to be causally related to vaccination.

Unsolicited AEs

The unsolicited AE profile was generally similar across groups (Table 5, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). Grade 3 (severe) unsolicited AEs were reported in 0.0% to 5.0% of subjects in any RTS,S/AS01_E group and in 0.0% of subjects in the control group (Table 6, Supplemental Digital

Content 1, <http://links.lww.com/INF/C993>). Two subjects reported unsolicited AEs considered to be causally related to vaccination: injection site swelling (6-, 10- and 26-week group) and gastroenteritis (≤7-day and 10- and 26-week group). No subject was withdrawn because of an AE post RTS,S/AS01_E vaccination. Unsolicited AEs within 30 days post-neonatal vaccination were reported in 21.8% and 27.5% of infants receiving RTS,S/AS01_E or a hepatitis B vaccine, respectively (Table 7, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>); none was considered to be causally related to vaccination.

Meningitis occurred in 2 infants: neonatal meningitis with no etiologic diagnosis in one subject in the ≤7-day, 10-week and 14-week group, which was reported 7 days post-dose 1 of RTS,S/AS01_E and pneumococcal meningitis in one subject in the 10-, 14- and 26-week group reported 8 days post-dose 1 of DTPw-HepB/Hib + OPV administered at 6 weeks of age without RTS,S/AS01_E co-administration. Neither event was considered to be related to

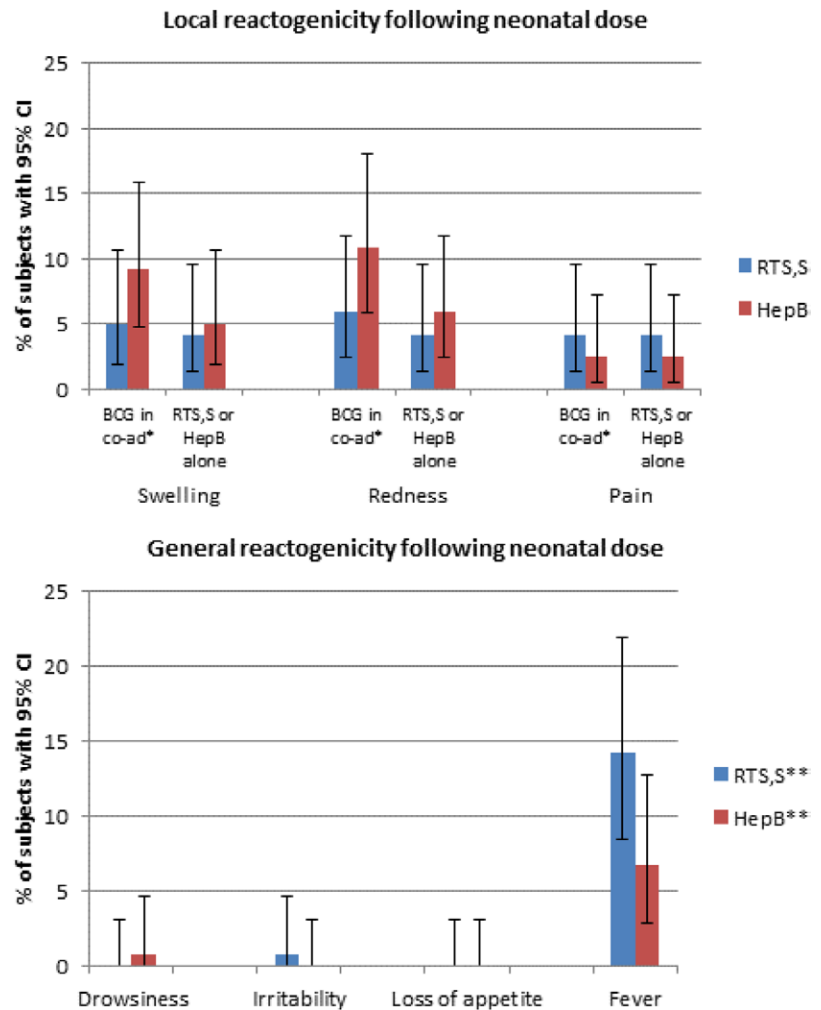


FIGURE 4. Bar graph of solicited local and general symptoms reported during the 7-day (days 0–6) postvaccination period after neonatal dose of RTS,S/AS01_E or a hepatitis B vaccine; pooled neonatal groups (Total Vaccinated Cohort). HepB indicates Pooled neonatal HepB vaccine groups (HepB vaccine at ≤7 days, RTS,S/AS01_E at 6, 10 and 26 weeks and Control [HepB vaccine at ≤7 days] groups); RTS,S, Pooled neonatal RTS,S groups (≤7 days, 10, 14 weeks and ≤7 days, 10, 26 weeks groups); *Combined local reactogenicity of RTS,S/AS01_E or HepB vaccine and BCG vaccination. **Combined general reactogenicity of RTS,S/AS01_E or HepB vaccine and BCG vaccination.

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vaccination and both resolved without sequelae. No case of generalized convulsive seizure within 30 days of vaccination was reported in any study group.

Reactogenicity

The incidence of solicited local AEs (pain, redness, swelling) and solicited general AEs of drowsiness, irritability and loss of appetite was generally similar in the RTS,S/AS01_E groups and the control group and similar after a neonatal dose of RTS,S/AS01_E or a hepatitis B vaccine (Tables 8, 9, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>; Figs. 2, 3, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). There was a higher frequency of fever (temperature ≥37.5°C) in most of the RTS,S/AS01_E groups compared with the control group and after a neonatal dose of RTS,S/AS01_E compared with a neonatal dose of a hepatitis B vaccine (Fig. 4, Table 9, Supplemental Digital Content 1, <http://links.lww.com/INF/C993>). Grade 3 fever (temperature >39.0°C) was reported in three subjects after dose 1 of RTS,S/AS01_E and in a further 2 subjects after dose 3 of RTS,S/AS01_E. No grade 3 fever was reported after a neonatal dose of RTS,S/AS01_E. No grade 3 fever was reported in the control group.

Clinical Laboratory Evaluations

No grade 4 abnormalities (potentially life-threatening) in biochemistry values were reported. Low hemoglobin or platelet

values were graded 4 in 5 subjects in the RTS,S/AS01_E groups, 2 after a neonatal dose of RTS,S/AS01_E and 1 subject in the control group, after a neonatal dose of a hepatitis B vaccine; none of the subjects had any relevant general medical history nor associated AE, and none of the grade 4 values was considered related to vaccination by the Investigator or by the Independent Data Monitoring Committee. These subjects all continued in the trial and completed the study procedures in good health.

DISCUSSION

This study has shown that superior anti-CS antibody levels were achieved after administration of the first dose of the RTS,S/AS01_E malaria vaccine to Malawian infants at 10 weeks of age on a 10-, 14- and 26-week schedule, compared with that at 6 weeks of age on a 6-, 10- and 14-week schedule. This first study to include a neonatal dose of RTS,S/AS01_E demonstrated an acceptable safety profile, but a reduced immune response.

In the pivotal phase III clinical trial of RTS,S/AS01_E, approximately 15,000 infants 6 to 12 weeks and children 5 to 17 months of age at first vaccination were enrolled across 11 sub-Saharan African sites. Administration of 3 doses of RTS,S/AS01_E 1 month apart resulted in protection against clinical malaria of 45% in the 5- to 17-month age group and 27% in the 6- to 12-week age group during 18 months' follow up after the third dose.⁹ Anti-CS

antibody titers were lower in the younger age category in which the mean age at first vaccination with RTS,S/AS01_E was 7.1 (standard deviation 1.4) weeks compared with 10.6 (standard deviation 3.8) months in the older age group.¹⁷

In the current study, all 7 RTS,S/AS01_E co-administration schedules yielded anti-CS antibody responses, which persisted to month 18.

Of note, infants who received RTS,S/AS01_E above 6 weeks of age as opposed to at 6, 10 and 14 weeks of age had an increase in post-dose 3 anti-CS antibody GMC, which was statistically significant for the 10-, 14- and 26-week group.

Lower anti-CS antibody responses were observed in infants receiving their first dose within a week of birth (≤ 7 -day and 10- and 14-week and ≤ 7 -day and 10- and 26-week schedules), with significantly lower titers compared with vaccination at 6, 10 and 14 weeks of age for the ≤ 7 -day and 10- and 14-week group. In the neonatal vaccine schedules, RTS,S/AS01_E was co-administered with only 1 or 2 doses of EPI vaccines compared with all 3 doses in the 6-, 10- and 14-week reference schedule, possibly indicating that the maturity of the immune system or inhibition because of maternally acquired antibodies, rather than an inhibitory co-administration response, could be implicated in the observed reduction in anti-CS titers compared with older children.¹⁸

Consistent with previous findings, there was no indication that increasing the spacing of the third dose of RTS,S/AS01_E was associated with higher anti-CS antibody responses,¹⁹ contrary to responses to hepatitis B vaccination in which improved anti-HBs antibody responses have been observed using this approach.^{10,11} Earlier studies reported enhanced anti-CS antibody responses post RTS,S/AS01_E vaccination in subjects who had previously received a dose of hepatitis B vaccine as part of EPI before study participation.^{13,14} However, in this study, anti-CS responses were similar when RTS,S/AS01_E was administered at 6, 10 and 26 weeks of age, with or without neonatal hepatitis B vaccination.

An exploratory schedule of RTS,S/AS01_E outside the EPI DTPw-HepB/Hib vaccination visits, in which the first dose was given at 14 weeks of age in co-administration with the third dose of DTPw-HepB/Hib, the second dose at 6 months of age without co-administration but correlating with a Vitamin A EPI visit and the third dose at 9 months of age in co-administration with measles vaccination, produced high post-dose 3 anti-CS antibody responses. Although GMCs for anti-measles antibody responses tended to be lower in the RTS,S/AS01_E co-administration group, seropositivity rates were similar with or without RTS,S/AS01_E co-administration. Noninferiority of anti-measles seroconversion rates has previously been demonstrated for RTS,S/AS01_E co-administered with measles vaccine compared with measles vaccine given alone.⁷

High anti-HBs antibody titers after RTS,S/AS01_E vaccination compared with standard hepatitis B containing EPI vaccines were demonstrated for all 7 schedules investigated. All subjects receiving 3 doses of RTS,S/AS01_E maintained seroprotective levels of anti-HBs antibodies over an 18-month duration. In contrast, anti-HBs seroprotection rates in subjects receiving a hepatitis B vaccine as a neonatal dose followed by 3 doses of DTPw-HepB/Hib decreased to 88.1% at month 18.

RTS,S/AS01_E was well tolerated in infants 1 to 7 days of age at first vaccination, with no SAE assessed as being causally related to vaccination. No subject was withdrawn because of an AE post RTS,S/AS01_E vaccination over the 18-month surveillance period of the study. In this first study of neonatal administration of RTS,S/AS01_E, the reactogenicity profile of a neonatal dose was comparable to that following vaccination of infants 6 weeks of age or older. As has been previously observed with RTS,S/AS01_E vaccination, transient mild to moderate fever occurs more frequently in RTS,S/AS01_E groups than in comparator groups.^{18,20}

Since initiation of enrollment to the current study in January 2011, the candidate RTS,S/AS01_E malaria vaccine has progressed to regulatory review. In 2015, the European Medicines Agency adopted a positive scientific opinion on the benefit–risk balance of RTS,S/AS01_E.^{21,22} Subsequently, the World Health Organization recommended pilot implementation of RTS,S/AS01_E vaccine in children as a 4-dose schedule in sub-Saharan Africa, covering moderate-to-high transmission settings: the first dose to be administered as soon as possible after reaching 5 months of age, the subsequent 2 doses after a minimal interval of 4 weeks and a fourth dose at 15 to 18 months after the third dose.²³ However, depending on malaria transmission, a substantial amount of malaria episodes can occur before the age of 5 months and an effective malaria vaccine that could be given together with some of the well-established EPI vaccination visits would be of added value to malaria control efforts.

The results of the present study indicate that first administration of RTS,S/AS01_E in co-administration with EPI vaccines at a slightly older age improves anti-CS immune responses. We note that this was a phase II study, with a small sample size, and as a consequence, results are descriptive rather than confirmatory since we have relatively low power to differentiate statistically between the groups for immunology and safety end points. Although no correlate of protection has been established, a link between higher anti-CS titers and decreased risk of infection has been shown in the challenge model and Active Detection of Infection trials,^{4,6,24–28} but not in Active or Passive Case Detection trials.^{5,8} The trial did not have an efficacy end point, and in the absence of a correlate of protection, the potential effect of the assessed schedules on malaria reduction could not be measured. Nevertheless, this study shows the potential to optimize immune responses to RTS,S/AS01_E by changing the immunization schedules, thereby potentially allowing for flexibility and alignment with health care delivery programs. More evidence is required on the feasibility of co-administration, and potential benefit in terms of efficacy, of RTS,S/AS01_E vaccination at a later age within the EPI schedule.

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